positioned to extend their immunocytochemical studies to live cell imaging. Microtubule polymerization is a highly dynamic process, and the association of regulatory proteins such as APC to microtubules in various states of assembly and stabilization would show more clearly how the protein regulates microtubules. As the authors suggest, guidance cues and their mediators may regulate local microtubule dynamics via APC. This would be an exciting avenue to pursue. For example, live cell imaging could reveal spatial changes in EGFP-APC in growth cones and their association with microtubules that were growing or shrinking in response to application of various growth and guidance cues.

We are now just beginning to unravel the mysteries of how extracellular cues modulate the cytoskeleton to shape growth cone behaviors and regulate axon outgrowth. Now that microtubules have been identified as leading players, more members of the +TIPS family are certain to be identified and their functions studied in neurons. It is clear from both papers that a major unsolved problem is to understand exactly how and where microtubules become stabilized, since asymmetrical changes in the cytoskeleton underlie growth cone guidance. In the future, live cell imaging will be required to identify the role of candidate proteins in regulating dynamic interactions between actin filaments and microtubules in growing axons.

Katherine Kalil[1] and Erik W. Dent[2]
[1]Department of Anatomy
University of Wisconsin-Madison
Madison, Wisconsin 53706
[2]Department of Biology
Massachusetts Institute of Technology
Cambridge, Massachusetts 02139

Selected Reading

Buck, K.B., and Zheng, J.Q. (2002). J. Neurosci. *22*, 9358–9367.

Dent, E.W., and Gertler, F.B. (2003). Neuron *40*, 209–227.

Dent, E.W., and Kalil, K. (2001). J. Neurosci. *21*, 9757–9769.

Dickson, B.J. (2002). Science *298*, 1959–1964.

Huber, A.B., Kolodkin, A.L., Ginty, D.D., and Cloutier, J.F. (2003). Annu. Rev. Neurosci. *26*, 509–563.

Kalil, K., Szebenyi, G., and Dent, E.W. (2000). J. Neurobiol. *44*, 145–158.

Lanier, L.M., and Gertler, F.B. (2000). Curr. Opin. Neurobiol. *10*, 80–87.

Lee, H., Engel, U., Rusch, J., Scherrer, S., Sheard, K., and Van Vactor, D. (2004). Neuron *42*, this issue, 913–926.

Pollard, T.D., and Borisy, G.G. (2003). Cell *112*, 453–465.

Rodriguez, O.C., Schaefer, A.W., Mandato, C.A., Forscher, P., Bement, W.M., and Waterman-Storer, C.M. (2003). Nat. Cell Biol. *5*, 599–609.

Schaefer, A.W., Kabir, N., and Forscher, P. (2002). J. Cell Biol. *158*, 139–152.

Zhou, F.Q., Waterman-Storer, C.M., and Cohan, C.S. (2002). J. Cell Biol. *157*, 839–849.

Zhou, F.-Q., Zhou, J., Dedhar, S., Wu, Y.-H., and Snider, W.D. (2004). Neuron *42*, this issue, 897–912.

To Learn Better, Keep the HAT on

Long-lasting memories are known to require new transcription. Recent studies have highlighted a role for epigenetic alterations, including histone acetylation, in regulating gene expression. In this issue of *Neuron*, Alarcón et al. and Korzus et al. use two different mouse models of Rubinstein-Taybi syndrome to elucidate a role for the histone acetyltransferase activity of CREB binding protein (CBP) in long-term memory and plasticity.

One of the most remarkable features of long-term memory is its persistence. The finding that short-term memories involve covalent modifications of proteins, whereas long-term memories involve new RNA and protein synthesis, has focused attention on the possibility that these newly expressed genes serve to maintain the memory. Epigenetic alterations, leading to chromatin remodeling, have recently been recognized to play a central role in the regulation of gene expression. Such epigenetic mechanisms involve DNA methylation as well as histone modifications, which include acetylation or methylation of conserved lysine residues at the amino-terminal domains of histone tails. These modifications alter chromatin structure and make specific regions of the genome more or less accessible to the transcriptional machinery. The histone modification machinery, together with methyl-CpG binding proteins, also serves to interpret the state of DNA methylation by establishing inactive chromatin structures at heavily methylated DNA loci, leading to gene silencing. Disruption of epigenetic mechanisms causes aberrant activation or silencing of selective genes, resulting in "epigenetic diseases" such as ATR-X, Fragile X, ICF, Angelman, Rett, and Rubinstein-Taybi syndromes (Egger et al., 2004). Interestingly, most of these syndromes are associated with mental retardation, suggesting that the development and function of the central nervous system is particularly sensitive to epigenetic abnormalities. However, the underlying mechanisms linking epigenetic regulation and mental retardation remain elusive.

A specific role for epigenetic mechanisms (DNA methylation) in long-term memory was originally proposed on theoretical grounds (Holliday, 1999). Studies of long-term plasticity of *Aplysia* sensory-motor synapses have recently revealed a particular role for histone acetylation and chromatin remodeling, in which stimuli producing long-term facilitation or long-term depression of these synapses regulate histone acetylation at the promoter of the immediate early gene C/EBP (Guan et al., 2002). In this issue of *Neuron*, Alarcón et al. (2004) and Korzus et al. (2004) use two different mouse models for Rubinstein-Taybi syndrome (RTS) to demonstrate that histone acetylation is critically required for long-term potentiation, learning, and memory in vertebrates and that the memory deficiency associated with RTS is due to haploid insufficiency of the function of CREB binding protein (CBP), particularly the histone acetyl-transferase (HAT) activity of CBP. RTS is characterized by skeletal abnormalities and severe mental retardation. It is caused by

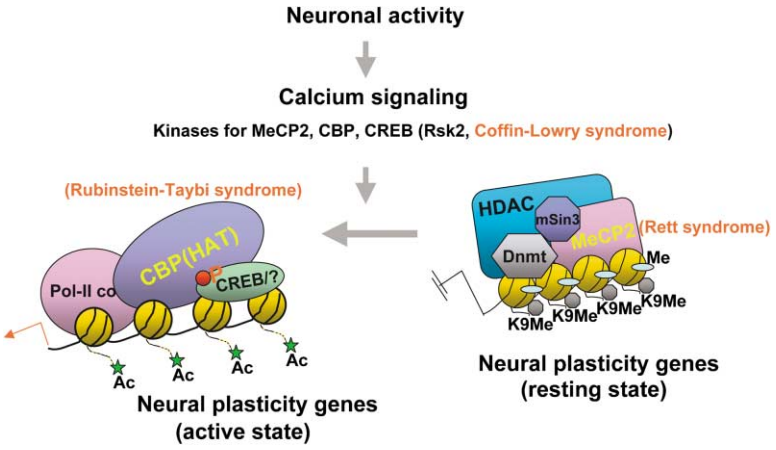

Figure 1. Neuronal Activity-Dependent Chromatin Remodeling Is Important for Neural Plasticity, Learning, and Memory

In resting neurons, neural plasticity genes (e.g., BDNF [Martinowich et al., 2003; Chen et al., 2003]) are associated with more inactive chromatin structures, in which histones are deacetylated or methylated on certain lysine residues (e.g., K9 of histone H3) and/or the DNA is more methylated (all of which are nonpermissive for transcription). Upon induction of neural plasticity, calcium signaling activates kinases for methyl-CpG binding protein, MeCP2 (mutations of which cause Rett syndrome), Rsk2 (a CREB kinase, mutation of which causes Coffin-Lowry syndrome), CREB, and CBP (mutation of which causes Rubinstein-Taybi syndrome). These events lead to a chromatin remodeling change in which transcriptional repression complexes dissociate from plasticity genes, transcriptional activators associate with these genes, histones become deacetylated, and the chromatin structure opens up, allowing for long-lasting expression of plasticity genes and long-term memory storage.

heterozygous mutations in the gene encoding CBP. The identification of *cbp* as the gene underlying RTS drew attention in the learning and memory community because it was consistent with a central role for CREB-mediated transcription in long-term memory. In addition to its role as a scaffolding/platform cofactor for CREB (as well as for other transcription factors), CBP acts as a histone acetyltransferase. Together, Alarcón et al. (2004) and Korzus et al. (2004) illuminate a central role for the histone acetyltransferase activity of CBP in long-term memory, providing strong support for the idea that chromatin remodeling serves to maintain memories.

Alarcón and colleagues based their studies on a previously generated mouse model for RTS, a conventional knockout of the *cbp* gene generated by Tanaka et al. (1997). While homozygous null mutant *cbp* mice die early during development (as do homozygous null humans), heterozygous ($cbp^{+/-}$) mutant mice exhibit phenotypes similar to certain clinical aspects of RTS. Behavioral studies in another mouse model of RTS, involving insertional mutation into the *cbp* gene leading to production of a truncated CBP protein, which functions as a dominant interfering form of CBP, had previously revealed deficits in long-term but not short-term forms of memory (Oike et al., 1999; Bourtchouladze et al., 2003). This type of mutation, however, is present in only 10% of patients with RTS, and studies in this mouse did not clearly differentiate between CBP's function as a scaffold/platform for other transcription factors and its HAT activity. Alarcón and colleagues thus chose to use the null allele heterozygous $cbp^{+/-}$ mice as a model for studying memory and plasticity deficits in the common haploid insufficiency form of RTS, and they used a variety of approaches to focus on the role of CBP's HAT activity in cognitive deficits.

Behavioral analyses revealed that $cbp^{+/-}$ mice exhibited normal levels of activity, motivation, anxiety, and working memory (a form of transient, short-term memory), despite the presence of significant deficits in motor learning, which are most likely due to abnormal skeletal development. The major memory defect detected in $cbp^{+/-}$ mice was reduced long-term memory, as assayed in contextual and cued fear conditioning and novel object recognition tasks. In contrast, no deficits in spatial learning were detected in $cbp^{+/-}$ mice using the Morris water maze. The authors speculated that repeated training in the water maze assay might overcome the memory deficits observed in tasks such as object recognition or fear conditioning, which are dependent on a one-time experience. The electrophysiological phenotype in the Schaffer collateral pathway of the $cbp^{+/-}$ mice revealed deficient late-phase long-term potentiation (L-LTP), which requires new gene transcription, but normal basal synaptic transmission and normal early LTP (E-LTP), which is independent of new mRNA and protein synthesis. No defect was observed in long-term depression of Schaffer collateral synapses. The defect in L-LTP was only partially ameliorated by enhancing CREB-dependent gene expression either by crossing the $cbp^{+/-}$ mice with transgenic mice expressing a constitutively active form of CREB or by administration of rolipram, an inhibitor of phosphodiesterase 4, which functions to increase signaling to CREB. These results suggest that memory deficits resulting from reduced CBP function reflect deficits in both CREB-mediated transcription as well as in CREB-independent gene activation. A histone deacetylase (HDAC) inhibitor, suberoylanilide hydroxamic acid (SAHA), was used to specifically address the role of histone acetylation in L-LTP and memory storage. Application of SAHA to hippocampal slices from wild-type and $cbp^{+/-}$ mice increased L-LTP, restoring the level and duration of LTP in mutant mice to that observed in wild-type mice in the absence of the drug. This increase correlated with an increase in histone 2B acetylation (which was found to be reduced in $cbp^{+/-}$ mice). When infused into the ventricle, SAHA also improved the deficits in contextual fear conditioning in $cbp^{+/-}$ mice.

To cleanly dissociate potential developmental abnormalities from adult-specific defects and to directly address whether the HAT activity of CBP or the scaffold/platform function of CBP is important for the function of CBP in cognition, Korzus et al. (2004) generated a hippocampal CA1 and dentate gyrus-specific, tetracy-

cline-inducible transgenic mouse that expresses a mutant form of CBP that lacks HAT activity (CBP{HAT$^-$}) in a spatially restricted and temporally inducible manner. Behavioral analyses of the CBP{HAT$^-$} mice revealed deficits in long-term but not short-term recognition memory, tested by a visual-paired comparison task, as well as deficits in spatial memory, assayed using the Morris water maze (notably, these deficits disappeared with intensive training). In contrast, long-term contextual fear conditioning was intact. Importantly, the defects in recognition memory and spatial memory were reversible upon termination of transgene expression, suggesting that pharmacological manipulation of the histone acetylation state might provide a potential therapeutic approach to ameliorate RTS symptoms. Korzus and colleagues also demonstrated that administration of another HDAC inhibitor, Trichostatin A (TSA), rescued the memory deficit in CBP{HAT$^-$} mice.

Recently accumulating evidence suggests that epigenetic mechanisms including DNA methylation and histone modifications are actively involved in neural plasticity, learning, and memory via regulation of critical gene transcription necessary for these biological processes (see Figure 1). These studies begin to uncover some of the mechanisms underlying the association between epigenetic diseases and mental retardation. Future challenges include identifying the signaling cascades leading to changes in histone acetylation and identifying the genes whose transcription is regulated via histone acetylation. Although additional studies will be necessary to reveal the gene-specific and coordinated regulation of the transcription network underlying normal neuronal function, the studies from Korzus et al. (2004) and Alarcón et al. (2004) shed light on the potential new "epigenetic therapeutic" approaches, i.e., developing drugs that can alter DNA methylation as well as histone modifications, to treat mental retardation and even other neurological diseases such as Huntington's disease.

Kelsey C. Martin[1,2] and Yi E. Sun[1,3]
[1]Department of Psychiatry
and Biobehavioral Sciences
Neuropsychiatric Institute
[2]Department of Biological Chemistry
[3]Department of Molecular and Medical
Pharmacology
University of California, Los Angeles
695 Charles Young Drive South
Los Angeles, California 90095

Selected Reading

Alarcón, J.M., Malleret, G., Touzani, K., Vronskaya, S., Ishii, S., Kandel, E.R., and Barco, A. (2004). Neuron *42*, this issue, 947–959.

Bourtchouladze, R., Lidge, R., Catapano, R., Stanley, J., Gossweiler, S., Romashko, D., Scott, R., and Tully, T. (2003). Proc. Natl. Acad. Sci. USA *100*, 10518–10522.

Chen, W.G., Chang, Q., Lin, Y., Meissner, A., West, A.E., Griffith, E.C., Jaenisch, R., and Greenberg, M.E. (2003). Science *302*, 885–889.

Egger, G., Liang, G., Aparicio, A., and Jones, P.A. (2004). Nature *429*, 457–463.

Guan, Z., Giustetto, M., Lomvarda, S., Kim, J.H., Miniaci, M.C., Schwartz, J.H., Thanos, D., and Kandel, E.R. (2002). Cell *111*, 483–493.

Holliday, R. (1999). J. Theor. Biol. *200*, 339–341.

Korzus, E., Rosenfeld, M.G., and Mayford, M. (2004). Neuron *42*, this issue, 961–972.

Martinowich, K., Hattori, D., Wu, H., Fouse, S., He, F., Hu, Y., Fan, G., and Sun, Y.E. (2003). Science *302*, 890–893.

Oike, Y., Hata, A., Mamiya, T., Kaname, T., Noda, Y., Suzuki, M., Yasue, H., Nabeshima, T., Araki, K., and Yamamura, K. (1999). Hum. Mol. Genet. *8*, 387–396.

Tanaka, Y., Naruse, I., Maekawa, T., Masuya, H., Shiroishi, T., and Ishii, S. (1997). Proc. Natl. Acad. Sci. USA *94*, 10215–10220.

Posterior Parietal Cortex: Space…and Beyond

How do we decide how to react to a stimulus or event? To do so requires recognition of the stimulus itself as well as an appreciation of the context within which that stimulus is encountered. In this issue of *Neuron*, Stoet and Snyder report that neurons in the parietal cortex of monkeys can carry contextual information related to the rules that are relevant for solving a visual discrimination task.

In our interactions with the world, how do we select appropriate behavioral responses to the continuous stream of stimuli and events around us? Not only must we determine the identity of a stimulus, but we must also take into account the context in which that stimulus is encountered. For example, a ringing telephone would require different responses at home (answer the phone) than when dining in a restaurant (let the host or hostess get it). If we were unable to take such contextual cues into account when planning voluntary actions, every stimulus would lead to a highly predictable reflex-like response that could be highly inappropriate in certain situations. Fortunately, this is not the case for many species of animals, including humans and monkeys. Our actions are jointly determined by sensory stimuli, past experience with those stimuli, and the context in which they are encountered.

While much is known about how the brain processes and encodes visual stimuli, comparatively little is known about the neural representation of behavioral context (also known as rules or "cognitive set"). The representation of context or rules has long been known to involve the frontal lobes of the brain, particularly the prefrontal cortex (PFC). A classic test of PFC functioning is a card-sorting test called the Wisconsin card sorting task (WCST). In this task, subjects are asked to sort a deck of cards (each with shapes of various number, shape, and color) into several piles based on a rule (i.e., match the color of items on each card) that the subject has to figure out by trial and error. Once the subject has figured out the rule, the experimenter covertly changes the sorting rule (i.e., match the shape of items on each card) and leaves the subject to adjust to the new sorting strategy. Normal subjects are very good at performing this task. They quickly learn the currently relevant rule and can rapidly adapt their strategy when the rule is covertly